Application Serial No. 60/079769 entitled "Nanomolar, Non-Peptide Inhibitors of Plasmepsin," filed on March 24, 1998 and bearing Attorney Docket No. 02307Z-0085320, the teachings of which are incorporated herein by reference.

5 II. <u>EXAMPLE II</u>

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A. Assays

1. Preparation and Maintenance of Entorhinohippocampal Slice Cultures

Organotypic entorhinohippocampal cultures were prepared using the technique of Stoppini, et al., J. Neurosci. Methods, 37, 173-182 (1991). Briefly, the caudal pole of the cerebral hemisphere containing the entorhinal cortex and hippocampus were harvested from brains of 6-7 days old Sprague-Dawley rat pups under sterile condition. 400 µm horizontal entorhinohippocampal sections cut vertical to the long axes of hippocampus were obtained using a McIlwain tissue chopper in a cutting medium consisting of MEM (with Earle's salts, Gibco), 25 mM HEPES, 10 mM Tris Base, 10 mM Glucose, and 3 mM MgCl₂ (pH 7.2). Brain tissue explants were then planted onto 30 mm cell culture inserts (Illicell-CM, Millipore, Bedford, MA) that were placed in 6 well culture trays with 1 mL of growth medium (MEM with Hank's salts, Gibco, 20% horse serum, 3 mM glutamine, 25 mM HEPES, 5 mM NaHCO₃, 25 mM glucose, 0.5 mM ascorbate, 2 mM CaCl₂, 2.5 mM MgCl₂, 0.5 mg/L insulin, and penicillin, pH 7.2; Bi, et al., J. Comp. Neuro., 401, 382-394 (1998). The cultures were incubated at 35°C with a 5% CO₂-enriched atmosphere and fed every other day until use.

After 10-14 days in vitro, organotypic cultures were incubated with growth medium containing either 20 μ M N-CBZ-L-phenylalanyl-L-alanine-diazomethylketone (ZPAD; BACHEM Bioscience, Torrance, CA), a selective inhibitor of cathepsins B and L (Shaw and Dean, 1980), in 0.01% DMSO, 20 μ M chloroquine (Sigma) or vehicle alone for days as specified. To test the effect of EA-1 on the generation of hyperphosphorylated tau fragments found in neurofibrillary tangles in Alzheimer's disease and other tau pathology-related diseases, 1 μ M of EA-1 or 10 μ M of CEL5-172 were applied alone or together with 20 μ M ZPAD.

2. Immunoblotting

For western blot, entorhinohippocampal explants were collected and sonicated in 10 mM Tris-HCl buffer (pH 7.4) containing 0.32 M sucrose, 2 mM EDTA,